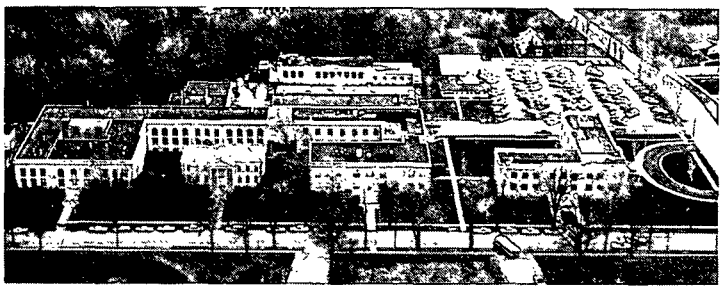

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THE PHYSICAL STRUCTURE OF MATURE *ACETOBACTER XYLINUM* PELLICLES

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NOTE TO THE EDITOR

The Physical Structure of Mature *Acetobacter xylinum* Pellicles

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Introduction

The formation of cellulose by *Acetobacter xylinum* has often been used as a model system for the study of cellulose biosynthesis. These studies have generally concentrated on the initial stages of fibril formation and have resulted in two primary hypotheses. In one, the cellulose is formed by extrusion of enzymatically synthesized glucan chains from the cell, followed by crystallization of adjacent glucan aggregates.[1] In the other hypothesis, cellulose is formed by the spontaneous crystallization of preexisting glucan molecules without any enzymatic action.[2]

The purpose of this note is to describe observations we have made of cellulose pellicles synthesized by *A. xylinum*, when we attempted to separate the individual fibrils for papermaking studies.

Experimental

The pellicles of cellulose were produced at pH 7, 28°C by two different batches of *A. xylinum* grown in a conventional medium[3] in 17 by 150 mm test tubes. The second batch was grown in media with either glucose or mannitol. Maximal yields were obtained in 18 days using an optimized surface-to-volume ratio. The first batch of pellicles was washed with water and dioxane, and treated with potassium hydroxide. A sugar analysis of pellicles from this first batch showed 92.2% glucose, 1.1% galactose, 0.9% mannose, 0.5% xylose, and 0.1% arabinose,

amounting to 94.8% of the material. The second batch was washed with only water. The pellicles were then solvent exchanged and freeze dried.

After storage, pellicles from the first batch were subjected to various treatments in order to separate the cellulosic fibrils, namely mechanical treatments, chemical treatments, and combinations thereof. These methods of separation included prolonged shaking in water, dimethyl sulfoxide, and alkanes; the use of blenders to beat the tissues; and crushing and milling to separate the fibrous elements. The pellicles were also digested in 1N sodium hydroxide and 1N sulfuric acid at 120°C.

The pellicles from both batches were cleaved for SEM examination in the horizontal and vertical planes using a freeze fracture technique where the fracturing is performed in liquid nitrogen.[4] The surfaces were shadowed with gold-palladium and examined using a JEOL JSM 35C scanning electron microscope operating at 15 kV (first batch) or 20 kV (second batch).

Results

All the attempts of separation were unsuccessful. Previous experiments where the pellicles were treated under a prolonged period of time with sodium chlorite using a method described by Thompson and Kaustinen[5] were unsuccessful, as well as the reaction with potassium superoxide[6] in dimethyl sulfoxide at room temperature followed by crushing actions. Our inability to separate significant quantities of cellulosic fibrils from pellicles using customary papermaking techniques suggested that very strong bonds existed between the fibrils. During the mechanical treatments, sheets of fibrils were infrequently torn from the pellicles but no fibrillation in the papermaking sense was observed.[7]

Pellicles were prepared for SEM examination using freeze fracturing techniques, since normal sectioning resulted in compression and distortion of the samples (see Fig. 1) from the first batch of pellicles. The external surfaces of the pellicles exhibited dense collapsed clusters of fibrils oriented in an irregular fashion (Fig. 2). Examination of the freeze fractured sections, on the other hand, showed an internal organization to exist within the pellicle.

Figures 1 and 2 here

Examination of the surface of the horizontally fractured pellicle showed bands which, at higher magnification, appeared to consist of layers of tunnels lying at angles to each other (Fig. 3 and 4). A higher magnification of a group of tunnels (Fig. 5), suggested diameters of about 7 μm. The reported dimensions of the bacterial cells are about 0.75 by 1.1 μm, which would give sufficient room for cells moving in the tunnels. The tunnels are woven from oriented cellulose fibrils and could be passageways caused by the movement of bacteria during cellulose synthesis.

Figures 3, 4, and 5 here

The fracture through the vertical plane of the pellicle shows a greater variety of tunnel orientations. The tunnels shown in Fig. 6 penetrate the plane of the picture in many directions. Although the sample is from the same specimen described above, the density of the fibrils about the tunnels appears higher in this instance.

Figure 6 here

The water-washed pellicles from the second batch contained bacterial cells which were absent from the pellicles described above. The cells were more abundant around the edges and on the upper surface of the pellicle (see Fig. 7). Cells with a fibril emerging from the end of the cell and dividing cells were observed. Bundles of straight fibrils loaded with cell clusters appeared on both the upper and lower surfaces of the pellicles (see Fig. 8).

Figures 7 and 8 here

The tunnels characteristic for the first batch of pellicles were not observed in the second batch. There was a tendency thereof as shown in Fig. 9, but it is lacking the high degree of organization seen earlier. These pellicles had a layered structure where they were more easily ruptured (see Fig. 10). The dark spots shown on the cross-section appeared, at a higher magnification, to be areas of less distinct fibrils with a more slimy character (see Fig. 11). These areas seemed to contain more cells than the surroundings.

Figures 9-11 here

Discussion

These observations support Colvin's contention[2] that the pellicles of A. xylinum have a three dimensional microfibrillar structure. However, the observations of the structure of the pellicle itself do not support his "brushwood" concept produced by the "association crystallization" hypothesis of microfibrillar formation. Instead, the observations can be better rationalized by assuming microfibrils to be produced from A. xylinum by the extrusion mechanism of Brown[8] and others.[9]

There are three main reasons why the results cannot be explained by Colvin's theory of microfibril formation. First, regularity and orientation of the structure argue against a random formation; second, the layered groups of tunnels can be explained with the bacterium building up the cellulose pellicle from the air-liquid surface; third, the existence of tunnels argues for some kind of coordination within a layer of the pellicle.

There are several uncertainties of these observations because the tunnel patterns are not exactly reproducible with different batches of bacteria. The influence of the growth environment and isolation technique are unknown. The inability to "beat" the cellulose can be related either to exceptionally strong hydrogen bonds between the fibrils or to intertwining of fibrils between different layers in the pellicle. The resistance to beating is related to the unexpected difficulty in measuring the viscosity[10] and the degree of polymerization of the cellulose. In addition, the reason for the resistance to degradation by potassium superoxide, acid, or base is yet unclear.

Many of the structural details in the micrographs are probably artifacts which result from the collapse of a more tenuous microfibrillar and aggregated microfibrillar architecture. This collapse (brought about by the isolation techniques) and the resultant formation of strongly hydrogen-bonded regions of the highly crystalline fibrils is likely responsible for the resistance of the pellicles to various mechanical and chemical treatments. Experiments are being undertaken to study these problems.

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Figure Captions

Fig. 1. The normal cutting of a pellicle (from the first batch) causes a compressed structure. Bar 100 µm, X100.

Fig. 2. The external surface of a pellicle (from the first batch) is dense and irregular. Bar 100 µm, X200.

Fig. 3. The horizontal cross section of a pellicle (from the first batch) shows layers of tunnels. Bar 100 µm, X100.

Fig. 4. The layers of tunnels are oriented in angles to each other (pellicle from the first batch). Bar 10 µm, X1000.

Fig. 5. The tunnels have an average diameter of 7 µm (pellicle from the first batch). Bar 10 µm, X3200.

Fig. 6. The tunnels in this vertical cross section are oriented in three directions (pellicle from the first batch). Bar 10 µm, X400.

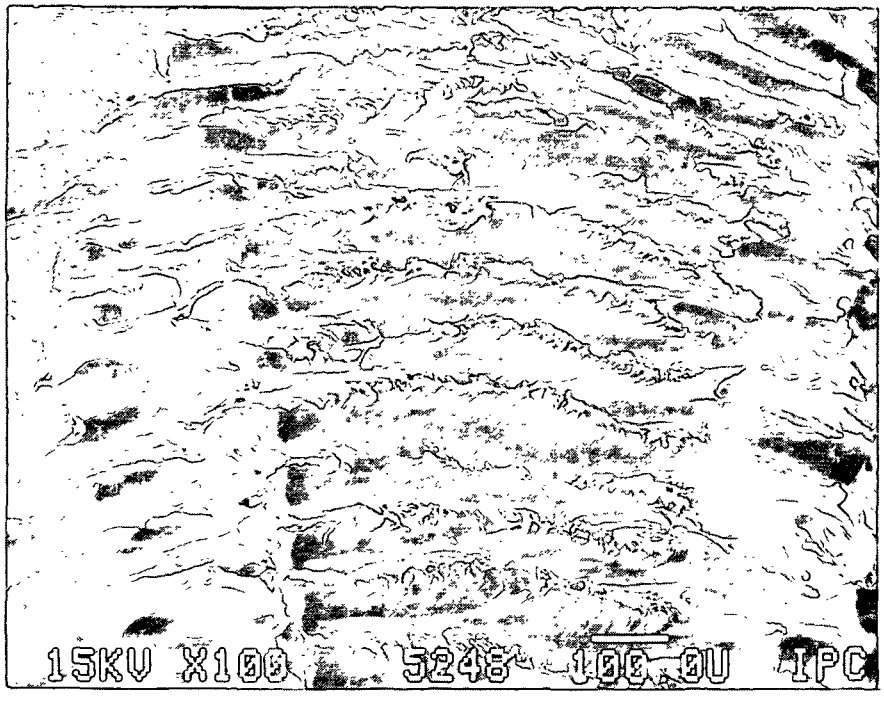
Fig. 7. The pellicles (from the second batch) were loaded with bacterial cells around the edges. Bar 10 µm, X3000.

Fig. 8. Bundles of straight fibrils appeared on the external surfaces of the pellicles (from the second batch). Bar 10 µm, X3000.

Fig. 9. The fibrils are deposited in circles, implicating tunnel formation. Bar 10 µm, X1000.

Fig. 10. The pellicles from the second batch have a layered structure. Bar 1000 µm, X24.

Fig. 11. Spots appeared on the cross section where the fibrils were less distinct (pellicle from the second batch). Bar 1 µm, X5000.



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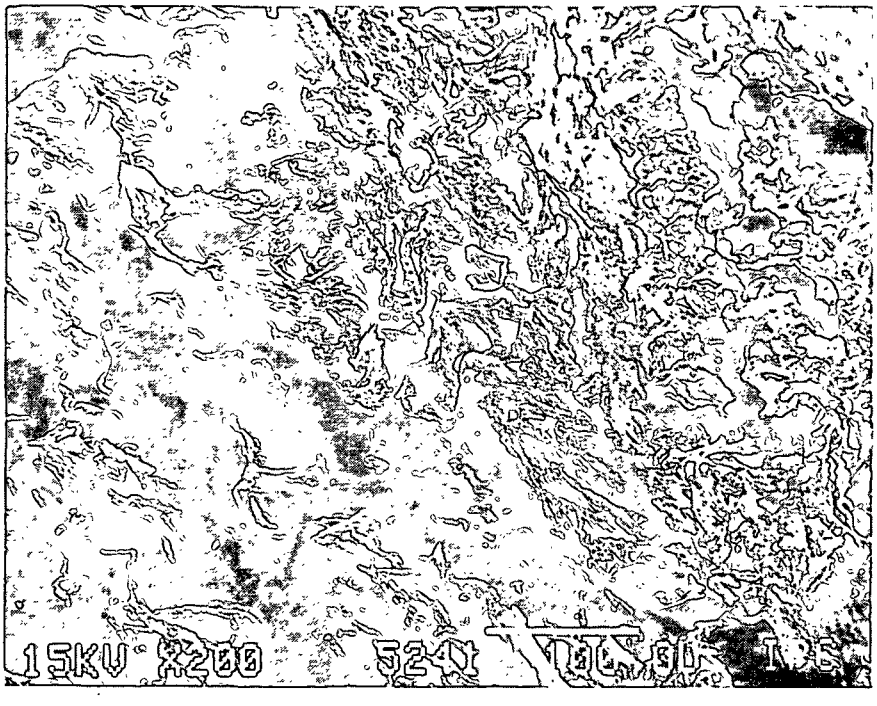

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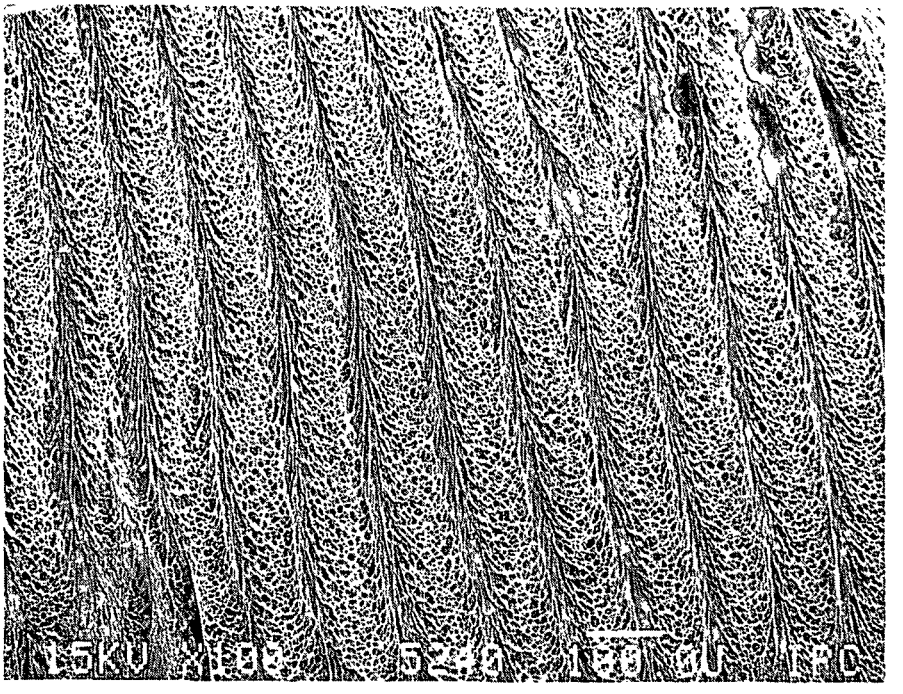

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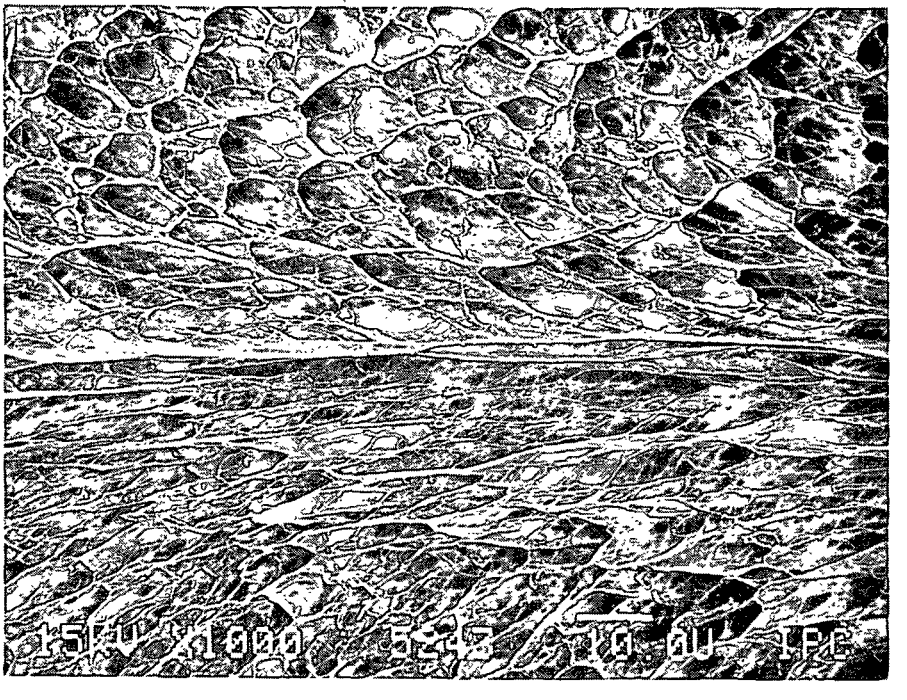

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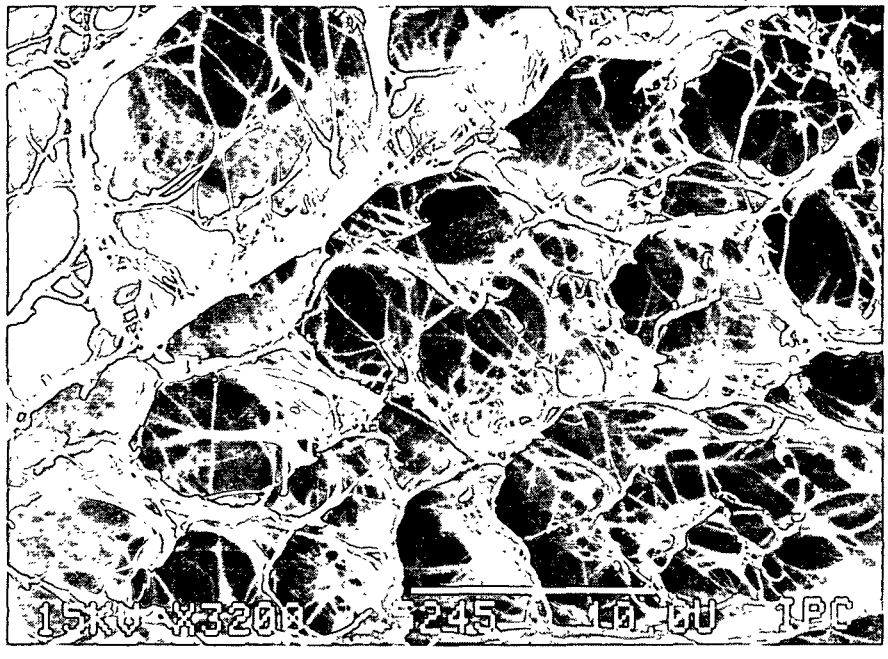

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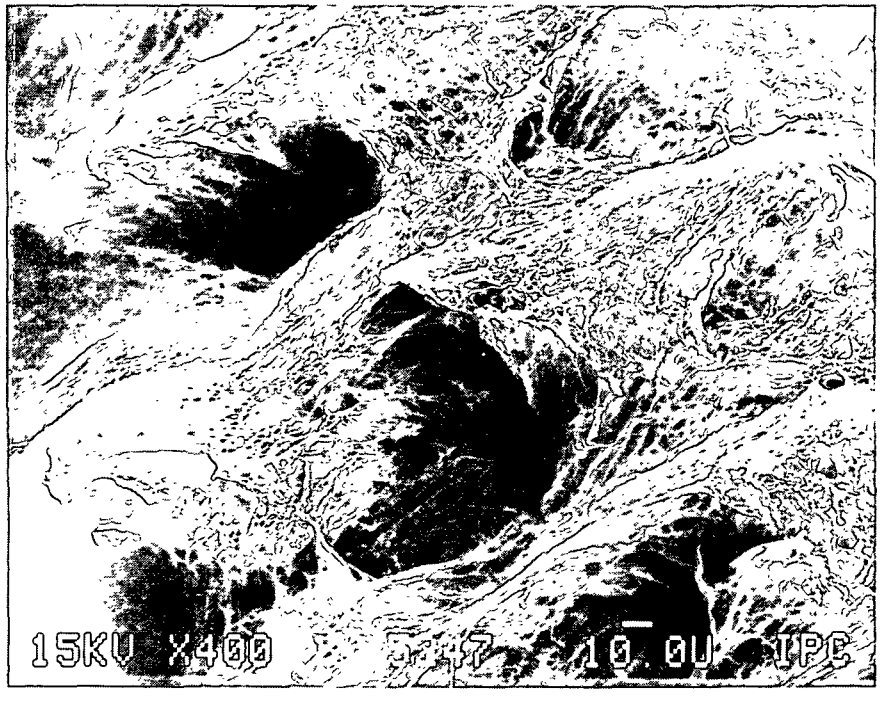

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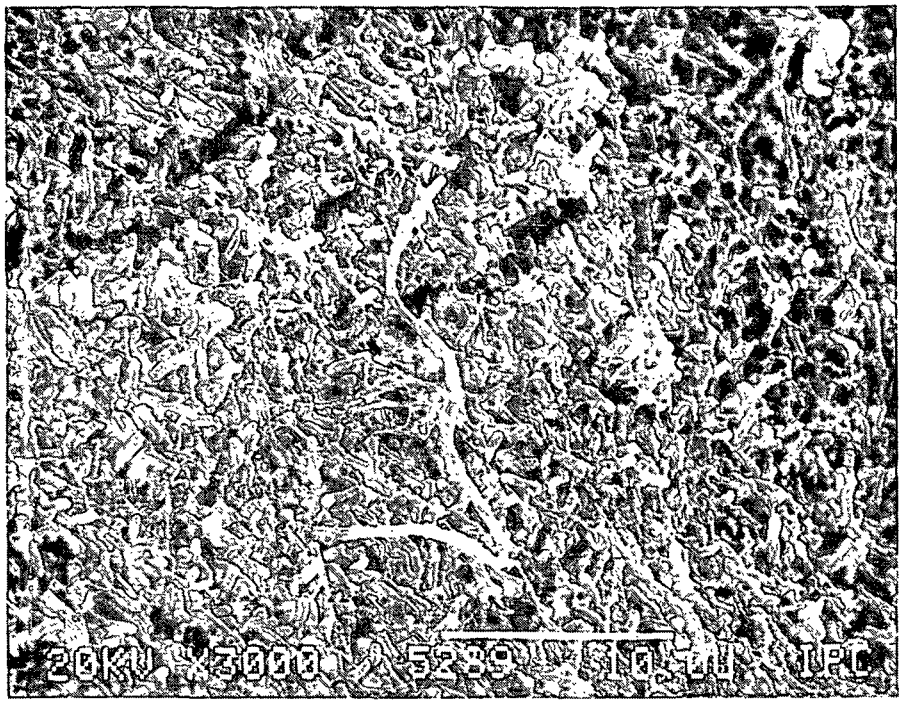

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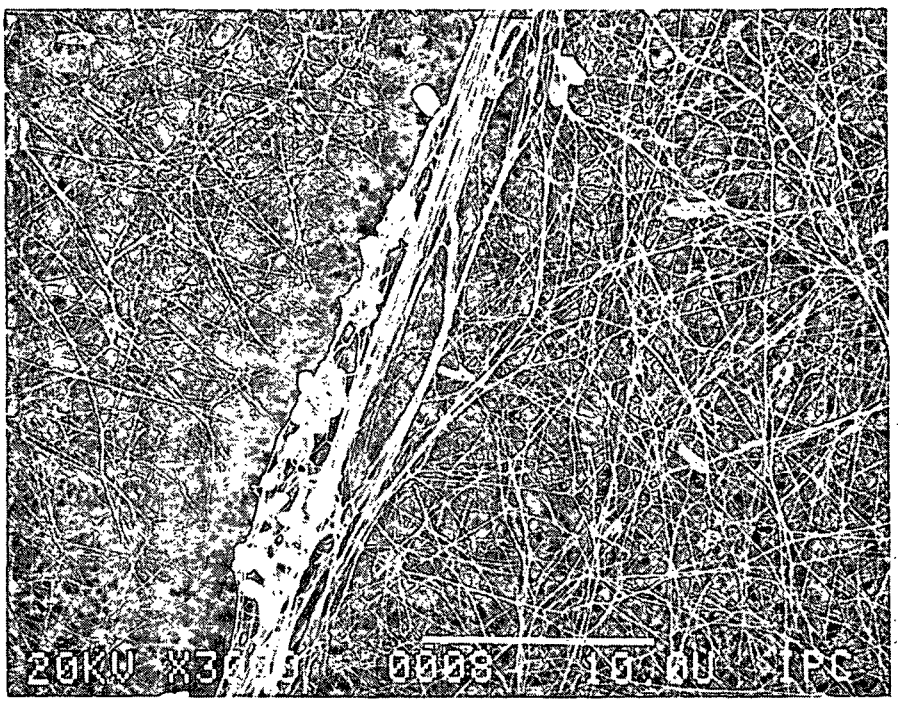

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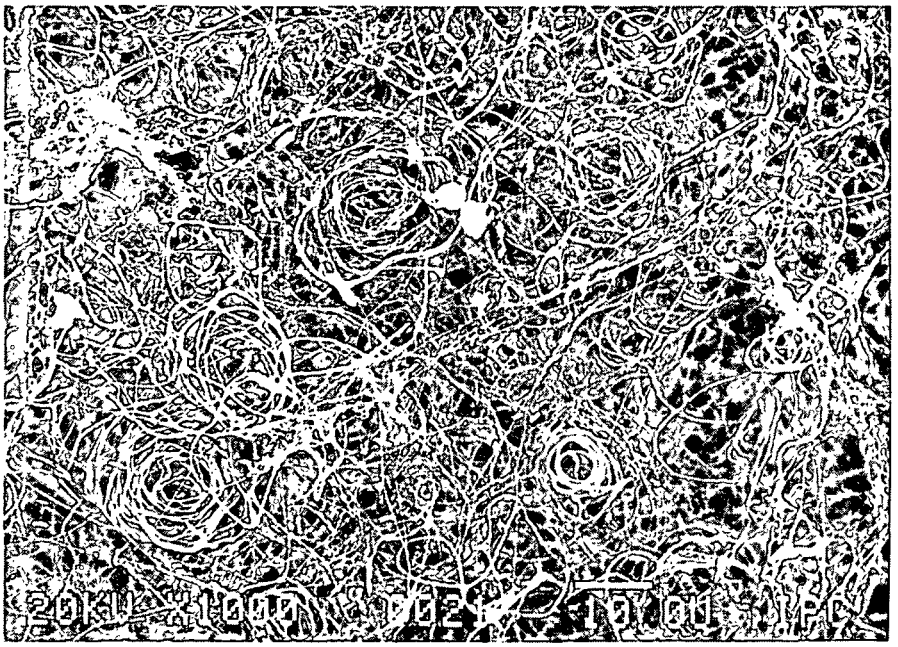

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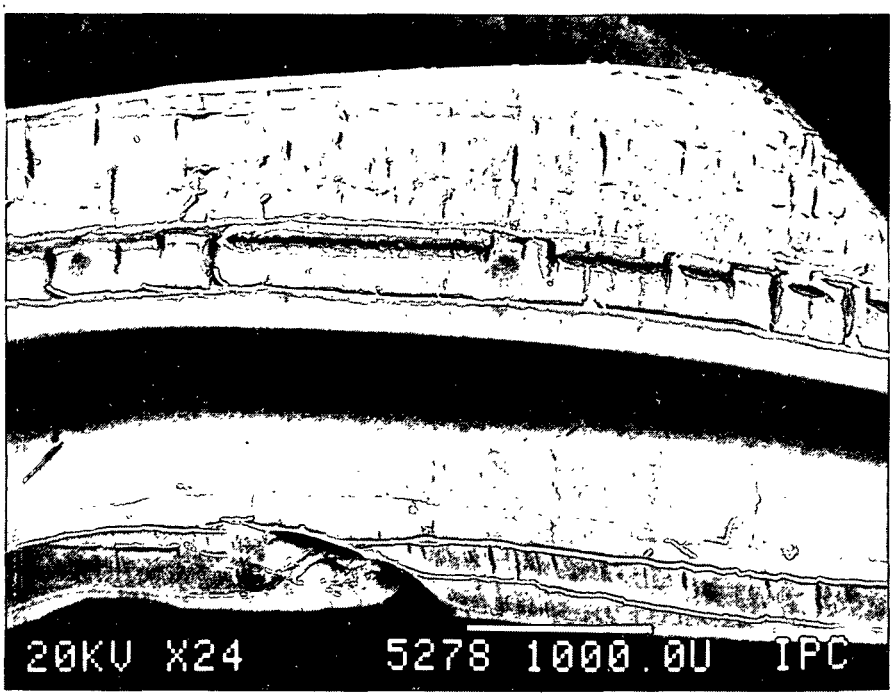

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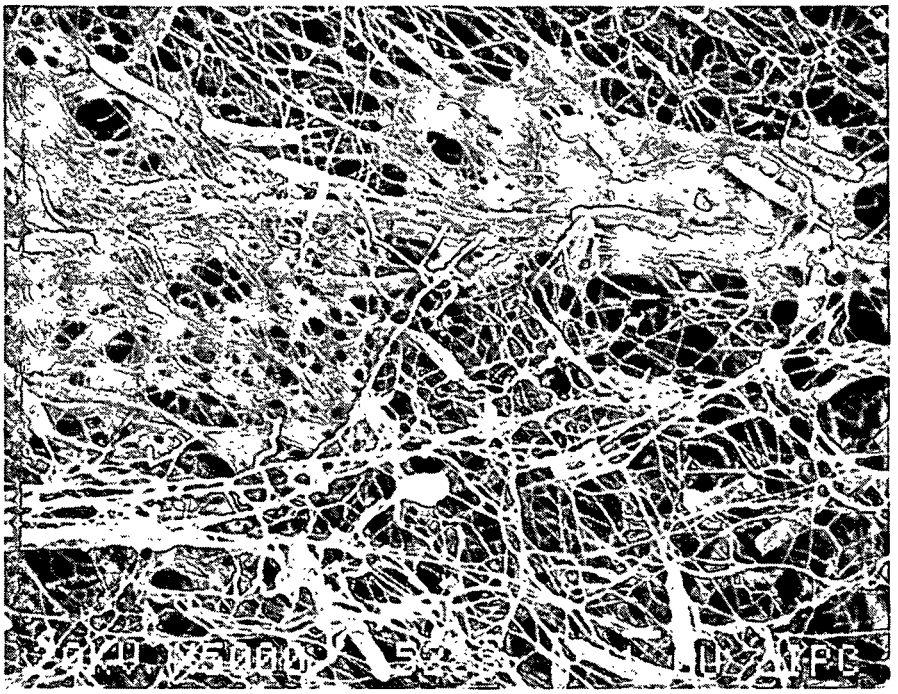

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